THE FREQUENCIES OF SEED AND SEEDLING ABNORMALITIES IN MAIZE¹

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THE fact that cross-fertilizing species carry relatively large numbers of deleterious recessives in a heterozygous condition has been demonstrated by various investigators and for several species. The results obtained have been used to make inferences or to provide a critical interpretation relative to population structure and size, types of gene action, mutation rates, selective advantage, and other attributes which contribute to the description of a population in genetic terms. Relatively little information is available, however, for the subsequent behavior of populations that have been experimentally purged of deleterious recessives. The purpose of the present paper is to provide information about one such case in maize.

Information on the frequency of deleterious recessives is most extensive for *Drosophila* species. Less information is available for many other species of animals and plants. We shall be concerned here with only the pertinent information available for maize.

JENKINS (1924) reported a range of 0.0 to 35.5 percent for chlorophyll-defective types in first selfed-generation progenies derived from 16 open pollinated varieties. In samples representing six varieties, HAYES and BREWBAKER (1924) reported frequencies of chlorophyll-deficient types ranging from 10.0 to 39.4 percent.

WENTZ and GOODSELL (1929) sampled 19 varieties which had been included in the Iowa State yield test for 1924 and 1925. The observed range for percentage of chlorophyll deficiencies was 3.6 to 42.8. In three varieties, WOODWORTH (1929) observed the percentage of self-pollinated ears segregating for chlorophyll deficiencies to range from 10.0 to 28.9. In a later summary WOODWORTH and MUMM (1931) reported 17.7 percent of self-pollinated ears of the variety Reid segregated for chlorophyll deficiencies and 3.9 percent for defective seeds. This report is of particular interest since a reconstituted Reid was produced by compositing remnant seed of all selfed ears whose progeny were free from detectable segregations. This reconstituted variety was propagated under open-pollination

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conditions for a three-year period. At the end of this period the reconstituted variety was sampled and the observed frequencies of chlorophyll deficiencies and defective seeds were found to be 28.6 and 12.2 percent, respectively. These figures represent nearly a twofold increase in chlorophyll deficient types and a threefold increase in defective seeds. No tests for allelism were made in either sample; therefore no estimates of gene frequency were obtained, and increase in frequency of seed and seedling segregations would not have been predicted. The present study was initiated to determine the generality of these results.

MATERIALS AND METHODS

A strain of the variety Reid, maintained by open pollination at the Iowa Agricultural Experiment Station, was used as source material for this study. In a special planting made in 1948 all plants which silked and tasseled at the same time were self-pollinated. Ears from a few plants were lost because of loss of or damage to the shoot bag or because of disease. The 801 self-pollinated ears available for study were assumed to constitute a random sample of the variety.

Each of the self-pollinated ears was shelled individually and a 50 kernel sample grown in a sandbench and examined for seedling segregations. Field plantings were made for all progenies classified as segregating. S_2 seed was obtained to confirm the tentative classification and to provide adequate material for subsequent tests for allelism.

Tests for allelism were made by direct intercrossing of the viable recessive types and classification of the resulting F_1 progeny. Progenies carrying lethals were grown in paired rows involving all possible combinations among similar phenotypes. Within each pair of rows as many intercrosses were made as stand and similarity of time of flowering would permit. With a monogenic character, 4/9 of such matings should exhibit segregation if plants of the two stocks were AA and Aa in expected frequency and carried members of the same allelic series. If positive indication of allelism was obtained, no further plantings were grown. If no segregation was observed, plantings were repeated until at least ten crossed progenies were available for classification.

After the initial classification for segregation was completed a reconstituted variety was prepared representing equal quantities of seed from the approximately 550 S_1 ears for which no segregations could be detected. Planting was made in an isolated plot and open pollination was permitted. Approximately 400 ears, chosen at random, were used to perpetuate the "reconstituted" variety. The ears were shelled in bulk and passed repeatedly through a sampler to ensure thorough mixing, and an aliquot was taken for planting a 1-acre or larger block the following season. This process was repeated during each of the four years during which the reconstituted variety was grown.

In 1953 approximately 1100 self-pollinations were made in the reconstituted strain. Seeds from the resulting ears were classified for seedling segregations and those classified as segregating were subjected to additional progeny tests for verification of the segregation and to increase seed for subsequent tests for allelism. Tests for allelism were conducted as described for the 1948 series.

EXPERIMENTAL RESULTS

In the sample from the original variety Reid, the percentages of self-pollinated ears segregating for seedling abnormalities was 18.9 (SCHULER 1952). This figure is in good agreement with percentages reported by other investigators. A list of the different phenotypic groups and their estimated gene frequencies is presented in Table 1. Estimated mutation rates are also presented for the nonviable mutant types.

TABLE 1

Estimated gene frequencies and mutation rates for the various mutant alleles isolated from the original Reid

Character	Allele	Frequency of occurrence	Observed gene frequency	Estimated mutation rate	Estimated μ/s equilibrium
Yellow-green					
Group 1	<i>yg</i>	a	2	.0013	1.5×10^{-6}
		b	1	.0006	$.38 \times 10^{-6}$
		c	1	.0006	$.38 \times 10^{-6}$
		d	1	.0006	$.38 \times 10^{-6}$
	2	e	5	.0031	9.7×10^{-6}
		f	3	.0019	3.5×10^{-6}
		g	1	.0006	$.38 \times 10^{-6}$
		h	1	.0006	$.38 \times 10^{-6}$
		i	1	.0006	$.38 \times 10^{-6}$
		j	1	.0006	$.38 \times 10^{-6}$
	3	k	10	.0062	38.9×10^{-6}
		l	1	.0006	$.38 \times 10^{-6}$
	4	m	1	.0006	$.38 \times 10^{-6}$
		n	4	.0025	6.2×10^{-6}
		o	2	.0013	1.5×10^{-6}
		p	2	.0013	1.5×10^{-6}
		q	1	.0006	$.38 \times 10^{-6}$
Luteus	<i>l</i>	r	1	.0006	$.38 \times 10^{-6}$
		s	1	.0006	$.38 \times 10^{-6}$
		a	7	.0044	19.1×10^{-6}
		b	2	.0013	1.5×10^{-6}
		c	1	.0006	$.38 \times 10^{-6}$
		d	1	.0006	$.38 \times 10^{-6}$
		e	1	.0006	$.38 \times 10^{-6}$
Stripe	<i>str</i>	a	3	.0019	3.5×10^{-6}
		b	1	.0006	$.38 \times 10^{-6}$
White	<i>w</i>	(a)	21	.0131	171.6×10^{-6}
		(b)	1	.0006	$.38 \times 10^{-6}$
Glossy	<i>gl</i>	3	4	.0025	6.2×10^{-6}
		13	1	.0006	$.38 \times 10^{-6}$
		(b)	1	.0006	$.38 \times 10^{-6}$
		(c)	1	.0006	$.38 \times 10^{-6}$
Dwarf	<i>d</i>	(a)	6	.0038	14.1×10^{-6}
		(b)	1	.0006	$.38 \times 10^{-6}$
Miscellaneous	.	.	1	.0006	$.38 \times 10^{-6}$
	.	.	1	.0006	$.38 \times 10^{-6}$
	.	.	1	.0006	$.38 \times 10^{-6}$

Two items require some explanation. Twenty-one ears of the 801-ear sample segregated for the virescent phenotype. Through accident this material was lost and no tests for allelism are available. Forty ears exhibited segregation for a yellow-green phenotype. Tests for allelism would have required 780 pairs of progeny rows. This number was greater than could be handled with facilities available. The material therefore was divided into four groups.

In an attempt to minimize the total number of crosses required Group 1 included all phenotypes which offered promise of viability. Allocations to the remaining three groups were at random. Intergroup crosses of established alleles were not completed. Thus gene frequencies for this phenotype may be slightly underestimated. Among the remaining phenotypes the estimated gene frequencies ranged from 0.0006 to .0131.

The estimates of mutation rates for all lethals are based on the assumption that the variety was at equilibrium; i.e. $\Delta q = 0$. Consequently $Q^2 = \frac{\mu}{s}$ and the mutation rate μ is equal to Q^2 since for all lethals $s = 1$. The mutation rates calculated in this indirect manner and under the assumption of equilibrium ranged from 0.38×10^{-6} to 171.6×10^{-6} .

In the sample derived from the reconstituted strain the frequency of seed and seedling abnormalities among 1,137 selfed progenies was 8.5 percent. This is approximately half the frequency obtained from the original variety sample. Estimates of gene frequency and mutation rates are presented in Table 2.

The estimated gene frequencies ranged from .0004 to .0101. This range was somewhat less than that observed for the Reid sample. Only ten different mutant phenotypes were observed in the Reid sample while 20 different phenotypes were recorded for the reconstituted Reid. Only six of these were common to the two samples. These except the luteus phenotype exhibit rather similar patterns of gene frequency as is illustrated in Table 3.

Tests for genetic identity among loci for the two samples were not attempted with all phenotypes. Within the glossy group, where the appropriate testcrosses were made, the similarity in frequency estimates does not extend to a complete duplication of loci involved. In the Reid sample gl_s was found four times, gl_{13} once and two additional unnumbered glossies once each. In the reconstituted Reid sample gl_s was found once, gl_{13} three times and gl_l was found nine times. This last glossy was not represented in the original sample.

DISCUSSION

One point of particular interest in this study and the earlier summary report of WOODWORTH and MUMM (1931) is the rapidity with which the reconstituted populations accumulated their mutation load. Several factors could conceivably be involved and these will be considered in the following section.

Sources of possible technical errors will be discussed first. All classification of mutants was either done or verified by the same individual. Thus personal bias in the recognition of aberrant types should have been at a minimum. For this

TABLE 2

Estimated gene frequencies, mutation rates, and equilibrium values for seed and seedling types isolated from the reconstituted Reid

Character	Symbol	Frequency of occurrence	Gene frequency	Estimated mutation rate	μ/s equilibrium
Luteus	<i>l</i>	1	.0004	1.6×10^{-7}	
White (1)	<i>w</i>	10	.0044	1.9×10^{-5}	
White (2)	<i>w</i>	1	.0004	1.6×10^{-7}	
Stripe (1)	<i>str</i>	1	.0004		1.6×10^{-7}
Stripe (2)	<i>str</i>	1	.0004		1.6×10^{-7}
Virescent	<i>v</i>	22	.0101		10.2×10^{-5}
Dwarf (1)	<i>d</i>	9	.0040		1.6×10^{-5}
Dwarf (2)	<i>d</i>	1	.0004		1.6×10^{-7}
Pale green (1)	<i>pg</i>	13	.0057		3.2×10^{-5}
Pale green (2)	<i>pg</i>	1	.0004		1.6×10^{-7}
Pale green (3)	<i>pg</i>	1	.0004		1.6×10^{-7}
Pale green (4)	<i>pg</i>	1	.0004		1.6×10^{-7}
Yellow green	<i>yg</i>	11	.0044		1.9×10^{-5}
Glossy	<i>gl₁</i>	9	.0040		1.6×10^{-5}
Glossy	<i>gl₃</i>	1	.0004		1.6×10^{-7}
Glossy	<i>gl₁₃</i>	3	.0013		1.7×10^{-6}
Shrunken	<i>sh₁</i>	17	.0074		5.5×10^{-5}
Onion leaf	..	2	.0009		8.1×10^{-6}
Sugary	<i>su₁</i>	1	.0004		1.6×10^{-7}
Adherent	<i>ad</i>	2	.0009		8.1×10^{-6}
Redleaf	..	1	.0004		1.6×10^{-7}
Red stripe	..	1	.0004		1.6×10^{-7}
Accessory blade	<i>ac</i>	2	.0009		8.1×10^{-6}
White leaf margin	..	1	.0004		1.6×10^{-7}

TABLE 3

Gene frequency ranges for six phenotypes common to the Reid and the reconstituted Reid samplings

Phenotype	Observed frequency ranges within			
	Reid	Reconstituted Reid		
Stripe	.0006	.0019	.0004	
Luteus	.0006	.0044	.0004	
Yellow-green	.0006	.0062	.0044	
White	.0006	.0131	.0004	.0044
Glossy	.0006	.0025	.0004	.0040
Dwarf	.0006	.0038	.0004	.0040

reason it is believed that the abnormalities detected upon sampling of the "re-constituted" Reid variety represent mutations occurring after the formation of this strain rather than undetected or latent segregations. The number of seed or seedling progeny examined from each self-pollinated ear was sufficiently large to provide odds of greater than 1000:1 that one or more mutant phenotypes should be detected if segregation was normal.

Aberrant segregations were of infrequent occurrence in either sample. The one exception to this generality was the class "germless" seeds. Because the limited progeny tests conducted were somewhat erratic, this class of segregants was ignored in both samples.

Exact records were not kept of the number of parental ears involved in each random mating generation. However, as a close approximation we can assume 550 ears for the composite forming the "reconstituted" variety, 400 ears to propagate the strain in 1950, 1951, and 1952, and 1137 ears representing the sampling in 1953. A total of approximately 6000 gametes was involved assuming two parental gametes per ear.

It is felt that the data obtained were influenced little, if any, by inaccurate classification, by failure to detect segregations, or by excessively small populations during the propagation phases. Consequently any genetic explanation for the observed results must involve one or more of the following: (1) a mutation rate materially greater than 10^{-5} , (2) a much larger number of loci than commonly assumed, or (3) a strong selective advantage for the heterozygote.

Generally speaking, the frequency of an allele giving rise to a deleterious or a normal phenotype is some function of its mutation rate and adaptive value and the breeding system involved. A stable equilibrium is eventually reached for lethals and for sublethals; this equilibrium value is simply the mutation rate μ . The equilibrium values for the two populations are given in Tables 1 and 2. The assumption of equilibrium is undoubtedly more realistic for the Reid variety than for the "reconstituted" Reid, which involved only four generations of free interbreeding. One may calculate the number of generations required to attain the observed frequencies in the "reconstituted" variety using the relationship

$n\mu = \log e \frac{p_0}{p_n}$ where n equals the number of generations to reach equilibrium,

μ equals mutation rate, and p_0 and p_n are the initial and final frequencies respectively. If we consider the allele, white (1), which was found ten times (Table 2), a very large number of generations are indicated as being required to reach this frequency. Either a high initial mutation rate or other undetermined forces must have been operative to achieve the observed frequency in four generations. A comparable situation was observed for other of the mutant phenotypes.

Tests for allelism of the glossy phenotypes were made within and between the two Reid samples as well as with known genetic stocks. Only two types, glossy 3 and 13, occurred in both samples but with differing frequency. Glossy 3 was present in greatest frequency in the Reid sample. If the difference was of a magnitude to be significant it would suggest that the reconstituted strain had failed to reach equilibrium. However the much greater frequency of glossy 13 in the reconstituted sample does not support such an argument. There remains a possibility that differences in mutation rate as well as in equilibrium may be involved. Either or both of these changes could conceivably have an effect on selective advantage.

If a selective disadvantage involving one or both of the homozygotes were

involved, inbreeding would increase the effectiveness of the selection. Assuming random mating and a minimum population size of 400 ears saved in each generation the expected rate of inbreeding would be approximately one percent per year. In the initial generation involving a composite of S_1 seed, however, assortive mating based on similarity in time of blooming may have led to somewhat greater inbreeding values. In any event it would appear that the inbreeding effects would be important only if accompanied by a selective disadvantage.

It is apparent from Tables 1 and 2 that the estimated mutation rates for the lethal phenotypes and the u/s equilibrium values for the viable types are of essentially the same order of magnitude. Such similarity suggests that if the heterozygote enjoys a selective advantage, such advantage could be rather similar for the two classes. It is assumed that in the reconstituted variety each of the loci in question was represented by the AA genotype until a mutation occurred. Following such a mutation, the frequency of the gene a in the next generation becomes

$\frac{q(1-sq)}{1-Sp^2-sq^2}$ where S and s represent the selective disadvantages of the AA and aa individuals, respectively. If the aa individuals are selectively eliminated as

would be true for a lethal, the preceding formula becomes $\frac{q}{1-Sp^2}$. Any se-

lective disadvantage of the AA type must result largely from a reduction in number of progeny rather than a differential viability. If a selective disadvantage of 0.5 is assumed and it is further assumed that the initial mutation occurred in one of the gametes produced by the 550 parental plants, then the a allele would increase about eightfold during the four generations involved in this study. If a mild selective disadvantage of 0.1 is assumed for the AA genotype, then the frequency of a would increase about 35 percent in the same period. Thus, while a strong selective disadvantage for AA could result in a rapid increase in the frequency of a , the fact that no such disadvantage has been demonstrated (SCHULER 1954; MANGELSDORF 1928; GARBER and ROWLEY 1927) renders this possible explanation for the observed results somewhat unattractive.

Experiments are currently underway to obtain additional information on the magnitude of any selective disadvantage. The longtime inbred line B14 is being used as the parental stock and induced mutations sought by use of UV and various chemical mutagens.

SUMMARY

Equilibrium values (μ/s) and mutation rates were determined for seed and seedling characters in the maize variety Reid. A reconstituted variety was established from S_1 seed from ears exhibiting no detectable segregation. After four generations of open pollination the reconstituted variety was sampled to provide new estimates for mutation rates and equilibrium values. The frequency of detected segregations was approximately half that of the original population. Mutation rates calculated for the original variety were not sufficiently high to account for the observed rate of accumulation of new mutations. A high initial mutation rate alone or in combination with a strong selective advantage for the

heterozygote could account for the observed results. The data available do not permit a critical distinction between these two alternatives.

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